INSTITUTE OF CHEMICAL BIOLOGY
UNIVERSITY OF SAN FRANCISCO
San Francisco, California 94117

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BRAIN AMINO ACIDS AND BIOGENIC AMINES UNDER VARIOUS ATMOSPHERIC MIXTURES

Arthur Furst and H. B. Chermside

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Arthur Furst, Ph.D.
Director

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INTRODUCTION AND SUMMARY

This semi-annual report covers the period from 1 Nov 66 through 30 April 67. Selected data from earlier reports are included for purposes of comparison and discussion.

The major concern of this project is the possible effect of artificial atmospheres upon brain biochemistry with emphasis on amino acids. After exposing rats to gaseous atmospheric mixtures for varying periods, the free amino acid pool and biogenic amine content (with emphasis on serotonin) of the animal's brains are measured.

The methodology is essentially the same as described in our earlier reports. For convenience, it is summarized in this report, and suggestions for improvements are made.

The concentrations of 40 ninhydrin-positive agents present in the free state in brain tissue of rats are reported. Six groups totalling 44 animals have been studied during this report period. These results are compared with previous results from 27 animals. Some conclusions are drawn from these data and further work is suggested. The number of test animals and exposures permits statistically significant conclusions.

The method for serotonin and catechol amines does not have the degree of sensitivity necessary to warrant drawing conclusions as to the effect of our exposure conditions on changes in these biogenic amines. Preliminary development of a more sensitive technique is reported.

We plan to continue analysis on more groups of animals in order to substantiate or refute the tentative conclusions we have reached. We plan also to explore other atmospheric mixtures of oxygen in combination with inert gases such as helium, neon, argon, or xenon as the major diluent compound.

EXPERIMENTAL PROCEDURE

GENERAL: Male rats of different strains were used - Long Evans and Sprague Dawley. These animals weighed between 350 and 500 g and were between 100 and 150 days old. The animals were exposed in our exposure chambers to the experimental or control condition desired for periods from 18 to 72 hours. They were then sacrificed and the whole brain removed within 60 seconds and stored frozen until prepared for analysis. Free amino acids were analyzed semi-automatically by ion exchange chromotography; biogenic amines were analyzed fluorometrically. The resulting data were processed variously on either of two computers and manually. A statistical analysis was made.

EXPOSURE: Exposure to experimental atmospheres were conducted in a chamber of our own design and construction. This chamber was reported in the previous semi-annual report dated 12 Dec 66, NASA publication N67-15883. The constitution of the experimental atmosphere mixture was controlled by the input metering system.

For exposure to 100% O_2, medical oxygen (Ohio Chemical & Surgical Equipment Co.) was used. The chamber was purged immediately after introduction of the animals, and then kept at a constant input flow rate of 0.5 ℓ/min controlled by (Ohio Chem & Mfgr.) metering valve. The chamber was vented to the atmosphere through a water trap with a head of 1 to 5 in to insure a pressure just sufficiently greater than ambient atmospheric pressure to prevent contamination from backflow of the surrounding atmosphere. The gases in the chamber were circulated through a purifying system at a rate of 5 to 7 l/min. This system included a cold trap liquid nitrogen (LN), conc. H_2SO_4, and dampened KOH pellets. It was observed that the LN cold trap apparently extracted all the biological waste H_2O, CO_2, and NH_3; the chemical system was a fail-safe backup to cover the contingency of blockage of the cold trap.

For exposures to 20% O_2+80% He (by volume) atmosphere the same equipment and technique were used with the following exceptions: Both medical oxygen and helium (Matheson, High Purity grade) were used. Input flow was controlled by a gas proportioner (Matheson Model 665) and total input flow was 5 l/min. The chamber was exhausted through a considerably larger manometer with a head of 2 inches of water. The cold trap of the purification system was immersed in a dry ice-acetone mixture, in order that liquification of the oxygen in the atmosphere not alter the proportions of the atmospheric constituents. There was no instrumentation for monitoring the atmosphere in the chamber; it was assumed that a flow rate of 5 l/min of the experimental gas mixture through the chamber (which has a volume of 13 L), would be great enough that the consumption of oxygen by a maximum of 10 rats would not significantly alter the ratio of oxygen to helium within the chamber from that prepared at the mixer.

All exposures were uninterrupted. Animals were allowed food and water *ad libitum* both before and during exposures.

TISSUE PREPARATION: After exposure, animals were sacrificed by decapitation with a guillotine (Harvard Apparatus) and the whole brain was removed. The olfactory lobes and brain stem were discarded and the brain was divided midsagitally. Each segment was then frozen in L N. For each animal this entire process took less than 60 sec. Each brain segment was individually wrapped in aluminum foil and stored in L N until it was prepared for analysis.

One half of each brain was prepared for analysis of free amino acids by a modification of the method of Talan, Moore, & Stein. The tissue was weighed and then crushed by a sharp blow while still frozen and wrapped. As much tissue as possible was transferred to a heavy duty 12 ml screw top centrifuge tube and homogenized in picric acid ultrasonically (Bronwill Biosonik II). The wrapping and surplus tissue were weighed. The homogenate was centrifuged and the supernatent transferred to a bed of Dowex 2X8 or 2X10. The precipitate was washed by rehomogenizing in H_2O; the mixture was then centrifuged again. This supernatent was also added to the Dowex bed. The total solution was washed from the Dowex bed into a 100 ml lyophilization jar with four washings of 0.02 N HCL. This solution, approximately 70 ml, was concentrated to a volume of approximately 4 ml by lyophilization and filtered through diatomaceous earth and an acid-washed Watman #1 filter paper into a small calibrated lyophilization jar. The filter bed was washed with H_2O until the volume in the small jar was approximately 15 ml. This solution was concentrated to a volume of 1 ml and then diluted to exactly 2 ml with a citrate buffer. It was then analyzed for concentrations of free amine acids. The special filters and calibrated lyophilization flasks were designed in our labs and were described in detail in our progress report of 9 May 66, NASA publication N66-26235.

The other half of each brain was prepared for fluorometric serotonin analysis. This was originally done by a modification of the method of Weisbach; in this preparation serotonin is extracted by differential solubility through agueous and alcoholic solvents. As this extraction has not proved successful in combination with the fluorometric technique used, it is being modified as discussed under Serotonin Analysis.

AMINO ACID ANALYSIS: The free amino acid content of the brains was analyzed on a Beckman 120-C amino acid analyzer following the method of Moore and Stein as modified in detail by Beckman and also by our group at the Institute of Chemical Biology. Of the 2.0 ml sample prepared, 0.5 ml was applied on each of two columns, one to analyze acidic and neutral, and the other to analyze the basic amino acids. Thus there was ample sample material to allow replication of any analysis in which there appeared to be an instrumental error. The charts from the recorder of the 120-C were evaluated by the dot-counting method. After the quantitative data were obtained, all other calculations were made by computer as described under COMPUTATIONS.

SEROTONIN ANALYSIS: The serotonin content of the brains was determined with an Aminco-Bowman spectrofluorophotometer. To 1.0 ml of the sample prepared following the method of Weisbach, 1.0 ml of concentrated reagent HCL was added and mixed in the special fluorescence cuvette. This solution was excited at a wavelength 295mμ and fluorescence occured at 540 mμ. Photometer readings were observed and recorded independently by two technicians. Determinations were re-run if these observations varied more than 1% of full scale reflection.

This procedure has not produced results we consider satisfactory. Both extraction and detection methods must be made more sensitive. A new technique for extraction has been recently published [WISE, Anal. Biochem 18, 94 (1967)] utilizing different solvents. A preliminary attempt has been made to use this method; results have been better than with the previous technique but as yet we are unable to get values as good as the results reported by Wise. It has been shown that serotonin can be treated with o-pthalaldehyde to increase fluorescence 20-100% [MAICKEL & MILLER, Anal. Chem. 38, 1937 (1966)]. A claim has been made that this reaction has been successfully used to increase fluorescence of extracted biological serotonin [MAICKEL, personal communications]. The application to biological material has not been published or accurately described, but some preliminary work on this has been done in this laboratory with promising results.

RESULTS

INTRODUCTION:

This portion of the report contains a presentation of the results of work accomplished during this report period. A comparison is also made with results from our earlier work. The first section lists conditions of exposure for each group of animals. This is followed by a section explaining the computations made. All data are tabulated and included in this report as an appendix; a short descriptive section is presented as the next section. The last section lists significant results found.

GROUPS:

Following the abbreviated title used for each group is a description of the exposure conditions for this group.

SD Cla: Five Sprague Dawley male rats 111 days old at sacrifice; "normal" controls taken from animal quarters with no special handling.

SD Clb:	Four Sprague Dawley male rats about 45 days old at sacrifice; "Ames controls" (controls for "Ames Exp") exposed to normal atmosphere at 760 mm Hg pressure in special exposure chambers at NASA Ames labs for 72 hr.
Ames Exp 1 & 2:	Two Sprague Dawley male rats about 45 days old at sacrifice exposed to 100% O_2 at 760 mm Hg for 72 hr in special exposure chambers at NASA Ames labs.
LE Cla:	Five Long Evans male rats 125-135 days old at sacrifice; "normal" controls taken from animal quarters with no special handling.
LE Clb:	Six Long Evans male rats 41 days old at sacrifice; "normal" controls taken from animal quarters with no special handling.
SD O_{100-18}	Eight Sprague Dawley male rats 113 days old at sacrifice exposed to 100% O_2 at 1 atm for 18 hr.
SD O_{100-50}	Three Sprague Dawley male rats 163 days old at sacrifice exposed to 100% O_2 at 1 atm for 50 hr.
LE O_{100-18}	Three Long Evans male rats 118 days old at sacrifice exposed to 100% O_2 at 1 atm for 18 hr.
LE O_{100-24}	Four Long Evans male rats 41 days old at sacrifice exposed to 100% O_2 at 1 atm for 24 hr.

LE O_{100-28} Six Long Evans male rats 117 days old at sacrifice exposed to 100% O_2 at 1 atm for 28 hr.

LE $He_{80}O_{20-18}$ Fifteen Long Evans male rats 90-105 days old at sacrifice exposed to a mixture 80% He, 20% O_2 at 1 atm for 18 hr.

Fluothane Exposures: Nine Long Evans male rats 113 days old at exposure; Exposure consisted of administration of Fluothane 3.6 mg/kg 40% solution in olive oil via stomach tube followed by exposure to 100% O_2 at 1 atm for 2.5 hr; these animals were sacrificed at various times after removal from oxygen exposure as follows: one at 0 hr, two at 24 hr, three at 48 hr, and three at 72 hr.

We are indebted to Dr. Henry Leon and Mr. Gerald Brooksby of NASA Ames labs for the opportunity to obtain the "Ames control" and "Ames Exp" immediately after exposure and to use their facilities for sacrificing the animals and preserving the brain tissue. All other animals were obtained from Simonson Labs, Gilroy, Calif. and quarantined in the animal quarters at the Institute of Chemical Biology for approximately one week before being exposed.

COMPUTATIONS: Some computations were made on an IBM 1620 from mark sense cards marked in the laboratory. Due to difficulties in obtaining time on this computer, an Olivetti Programma 101 desk top computer was later utilized. Although calculation time is considerably greater with this computer, there is no need for mark sense cards. It also allows immediate checking of suspected errors against the raw data. Therefore total computation time is not significantly increased, and it is felt that there is less chance for error.

The following values were calculated in relation to the amino acid: a) An instrumental constant for each standardized amino acid; b) the concentration in nanamoles per gram frozen brain weight (nM/g) for each ninhydrin-positive substance observed in each animal (for compounds without standardization this value was calculated in glutamic acid units); c) means ± 1s.d. for each substance over all animals in each group; d) Student's "t" ratio for each substance between each experimental group and the appropriate control group.

DATA:

Data are presented in Tables 1 through 14 which are appended to this report. Some of these tables contain mean values ± 1s.d. for each ninhydrin-positive compound found (concentrations are expressed as nanamoles per gram frozen brain weight, nM/g); others show student's "t" statistic computed for each experimental group and the appropriate control group, with significance noted where $P<0.20$.

The tables are arranged as follows:

Table 1: A list of ninhydrin-positive substances found, keyed to the numbers assigned to these compounds for use in other tables.

Table 2: Concentrations for group SD Clb ("Ames control") and for each "Ames Exp" animal.

Table 3: Concentrations for Fluothane exposure combined and broken into sub-groups by delay after exposure.

Table 4: Concentrations for the four control groups; SD Cla, SD Clb, LE Cla, LE Clb.

Table 5: Concentrations for Long Evans animals involved in 100% oxygen exposure experiments: LE Cla, LE Clb, LE O_{100-18}, LE O_{100-24}, LE O_{100-28}.

Table 6: Concentrations for Sprague Dawley animals involved in 100% oxygen exposure experiments: SD Cl, SD O_{100-18}, SD O_{100-50}.

Table 7: "t" for comparison between species: LE Cl, SD Cl.

Table 8: "t" between Le control and 18 hr O_2 exposure: LE Cl, LE O_{100-18}.

Table 9: "t" between LE control and 24 hr O_2 exposure: LE Cl, LE O_{100-24}.

Table 10: "t" between LE control and 28 hr O_2 exposure: LE Cl, LE O_{100-28}.

Table 11: "t" between LE control and 18 hr $He+O_2$ exposure: LE Cl, LE $He_{80}O_{20-18}$.

Table 12: "t" between SD control and 18 hr O_2 exposure: SD Cl, SD O_{100-18}.

Table 13: "t" between SD control and 50 hr O_2 exposure: SD Cl, SD O_{100-50}.

Table 14: "t" between controls and fluothane exposure: LE Cl, Fluothane total.

OBSERVED EXPERIMENTAL DIFFERENCES:

The following differences in concentrations of various ninhydrin-positive substances have been found for various groups of animals:

Strain Difference:

The concentration of Glycerophosphoethanolamine is greater ($P < 0.02$) in Long Evans rats than in Sprague Dawley.

The concentration of Isoleucine may be greater ($P < 0.20$) in Sprague Dawley rats than Long Evans.

Oxygen Exposure, 100% at 1 atm.:

For exposures of 18 hr, there were no significant differences observed in either species.

For exposures of 24 and 28 hr, both performed on Long Evans rats, few differences were found which may be considered reliable: Although tyrosine was possibly significantly different in both cases ($P < 0.20$), in one case O_2 exposure increased the concentration and in the other case exposure reduced the concentration. In one case ammonia was decreased significantly ($P < 0.002$), but, as instrumental determinations of ammonia are affected by the atmosphere in the laboratory, and as there was no difference in ammonia concentration in any other group, it is reasonable to assume that this difference is an artifact of the analytical process. Exposure to O_2 for 24 hr reduced the concentration of phosphoserine ($P < 0.02$), but exposure for 18 or 28 hr did not.

Exposure to 100% O_2 for 50 hours increased the concentrations of both glutamine and glutamic acid ($P < 0.002$, $P < 0.002$). This may be a real effect; as the exposed group was small (N=3), it is felt that this exposure should be replicated.

Helium-Oxygen exposure:

Exposure to an atmosphere 80% He 20% O_2 for 18 hr made a highly significant reduction ($P < 0.002$) in the concentration of phophoserine. No other differences were more than possibly significant ($P < 0.20$ or 0.10). Analysis of longer exposures to this mixture will permit better evaluation of these data.

Fluothane exposure:

Exposure of fluothane-pretreated animals to 100% O_2 for 2.5 hr lowered significantly ($P < 0.002$) the concentration of phosphoserine. No other changes were more than possibly significant ($P < 0.20$). These data are calculated from the combined values of all animals in the fluothane exposure group; the raw data will be further analyzed to determine if there was any effect due to the delay in sacrifice after exposure.

DISCUSSION OF RESULTS:

It is felt that these results are preliminary; they should be re-evaluated when data are available for a larger group of control animals and for longer exposures.

It was reported earlier (N67-15883) that exposure to 100% O_2 may change the concentrations of free pools of several amino acids. These changes were not statistically significant. Two effects of oxygen exposure do appear significant: exposure in most cases reduces the concentration of phosphoserine; long exposure increases the concentrations of glutamine and glutamic acid.

Strain differences appear to be non-significant. For this reason we will restrict our further experiments to a single strain - Long Evans.

FURTHER WORK PLANNED

The principle direction of further work will be toward examination of the effects of mixtures of oxygen and various inert gases. A beginning of this work was made with the helium-oxygen exposure. At the request of NASA we will work also with mixtures in which the inert component will be argon and neon; we will also make longer exposures in the helium-oxygen atmosphere.

Due to the cost of these inert gases, we cannot use our present constant loss exposure equipment. We are developing a slightly different system including: a chamber which can be better sealed; a demand oxygen supply system; an oxygen concentration monitor.

A new ion exchange resin has recently been released (Beckman Instruments) which will allow resolution of glutamine from asparagine, and reduce analysis time for acidic and neutral amino acids. We plan to utilize this resin.

We will continue work on development of a more sensitive measure of serotonin and catechol amines.

TABLE 1

Ninhydrin-position compounds observed in rat brain free amino acid pools -

Compound	Elution time,min	Identification[A] standard	Quantitative[B] standard	ICB control number
Acidic and Neutral Compounds				
Phosphoserine	23	?	G	2
Glycerophosphoethanolamine	27	?	G	3
Phophoethanolamine	31	?	G	4
Taurine	37	C	G	5
Urea	42	C	G	6
Unknown #1[C]	54	??	G	8
Unknown #2[C]	59	??	G	9
Unknown #3[C]	65	??	G	11
Aspartic Acid	74	C	S	12
Threonine	77	C	S	13
Serine	83	C	S	14
Glutamine[D]	89	?	G	15
Unknown #6	95	??	G	24
Glutamic Acid	123	C	S	17
Glycine	139	C	S	18
Alanine	147	C	S	19
Unknown #4	153	??	G	20
alpha-Aminobutyric Acid[E]	160	C	G	21
Valine[F]	186	C	S	22
Cystathionine	201	C	G	26
Methionine	207	C	S	28
Unknown #5	210	??	G	29
Isoleucine	213	C	S	30
Leucine	220	C	S	31
Tyrosine	249	C	S	34
Phenylalanine	256	C	S	35
beta-Alanine	283	?	G	36
beta-Aminoisobutyric Acid	305	?	G	37
Basic Compounds				
gama-Aminobutyric Acid	102	C	G	40
Ornithine	112	C	G	41
Ethanolamine	127	C	G	43
Ammonia[G]	134	C	S	44
Lysine	149	C	S	46
Histidine	182	C	S	48
Carnosine	235	C	G	50
Tryptophane	244	C	G	54
Argenine	329	C	S	56

FOOTNOTES TO TABLE 1

A. C=compared to known standard
?=tentative-agrees with published data
??=unknown

B. S=standardized values used for calculations
G=Glutamic Acid units used for calculations

C. Unknowns #1,2,3, (??#1, ??#2, ??#3)

Examination of the chromatograms for 71 animals indicates that the data presented regarding the ninhydrin-positive substances ??#1, ??#2, and ??#3 may be erroneous. It appears that ??#2 may sometimes be eluted with ??#1, sometimes with ??#3, and sometimes well enough separated from either to be observed as a separate substance. Our unsubstantiated opinion is that there are three separate ninhydrin-positive substances eluted in that portion of the anlysis, but that various parameters of the analytical process cause contamination of the data. These substances are present in the brain in low concentrations only, and until they are identified the importance they may play in brain metabolism under any conditions is unknown. For these reasons we do not plan to follow up with special research on these substances during the period of this grant unless fortuitous circumstances present us with more concrete information regarding them.

D. Glutamine

The identification of Glutamine is uncertain. We find that our Beckman 120-C when operated in accordance with the Beckman instruction manual based on the work of SPACKMAN, STEIN, and MOORE [Anal. Chem. 30, 1190 (1958)] will not resolve both glutamine and asparagine when they are present in amounts greater than approximately 0.05 µM in the analysis sample. The compound eluted from our brain samples at this point on the chromatogram is present in the sample in an amount on the order of 0.20 µM. We assume the tentative identifcation of this peak as exhibited in brain sample analysis to be glutamine rather than asparagine because of work report by SHAW and HEINE [J. Neurochem. 12 151 (1965)], by MUSSINI and MARCUCCI, and by TALLAN [both in Amino Acid Pools, J. T. Holden, ed., New York: Elsevier (1962)] and others. However, it is equally possible that both substances are present and cannot be differentiated by our analytic technique. It is hoped that a new resin may resolve these separately (see FURTHER WORK).

E. alpha-Aminobutyric Acid

SHAW and HEINE reported an unknown substance in rat brain tissue eluted between alanine and valine. We find two compounds in this area, one of which we have identified as alpha-Aminobutyric acid by comparison with a known standard. It should be noted that we use a different analytic

FOOTNOTES TO TABLE 1 cont'd.

instrument with different ion exchange resins and buffers of different pH, so we cannot state with certainty that we have identified the peak SHAW and HEINE reported as unknown.

F. Valine

The values reported for valine may include some cysteine. Our instrument does not always satisfactorily resolve cysteine and valine.

G. Ammonia

Values for ammonia cannot be considered accurate due to contamination of atmosphere in the laboratory.

table 2

AA#	SD C1b Ames Cont. nM/g N=4	s.d.	Ames Exp 1 nM/g N=1	Ames Exp 2 nM/g N=1
2	127	18	100	291
3	236	36	181	79
4	1352	1088	724	1002
5	1121	175	675	854
6	495	237	156	55
8	38	10	—	63
9	5	3	—	—
10	1	—	—	—
11	43	32	—	—
12	466	50	1990	2801
13	412	318	—	—
14	804	216	1135	1507
15	764	217	538	4115
17	1988	349	878	1610
18	851	106	692	1227
19	464	83	208	424
20	21	14		
21	2	1	—	—
22	85	11	93	—
25	4	8	—	—
26	64	26	37	109
27	2	2	—	—
28	26	14	4	52
29	6	4	—	—
30	30	2	40	39
31	67	10	84	79
34	67	16	44	58
35	39	6	41	119
36	11	2	—	—
37	29	8	—	—
40	1517	785	961	1632
41	26	3	119	56
43	99	34	122	282
44	1.205	177	2695	1139
46	225	43	375	339
48	55	7	116	11.6
50	61	2	10	84
54	80	10	52	75
56	141	12	100	93

table 3

Fluothane exposures:

AA#	0 nM/g N=1	24 nM/g mean	24 s.d.	24 N	48 nM/g mean	48 s.d.	48 N	72 nM/g mean	72 s.d.	72 N	Combined mean	Combined s.d	Combined N
2	12	19	5	2	21	3	3	43	5	3	27	13	9
3	88	137	16	2	113	47	3	165	21	3	133	38	9
4	126	280	45	2	228	24	3	389	16	3	282	94	9
5	1237	2190	313	2	2083	336	3	3006	316	3	2321	644	9
6	10	26	1	2	23	18	3	29	1	3	24	11	9
8	7	13	7	2	19	4	3	30	8	3	20	10	9
9	8	7	1	2	10	—	1	12	1	2	9	2	6
10	9	8	2	2	14	5	3	66	28	3	30	31	9
12	276	649	390	2	607	437	3	1437	483	3	857	572	9
13	248	362	106	2	240	190	3	521	98	2	342	168	8
14	402	479	60	2	560	163	3	619	32	3	545	115	9
15	1743	3030	415	2	2242	239	3	2713	32	3	2519	469	9
17	5090	2988	1007	2	3963	466	3	8087	568	3	5246	2277	9
18	436	649	77	2	765	95	3	586	51	3	639	124	9
19	230	312	18	2	310	35	3	300	39	3	299	38	9
20	8	13	—	1	54	10	3	5	1	2	28	26	7
21	1	1	—	1	4	—	1	2	1	2	2	1	5
22	42	52	—	1	29	28	3	48	4	3	41	18	8
23	6	15	—	1	13	—	1	1	—	1	9	7	4
26	15	22	—	1	23	3	3	31	8	3	25	7	8
28	3	18	—	1	30	3	3	18	2	3	21	10	8
29	1	2	—	1	5	2	2	7	1	3	5	3	7
30	15	25	—	1	32	4	3	19	2	3	24	7	8
31	46	64	—	1	80	5	3	48	2	3	62	16	8
34	6	21	4	2	32	7	3	28	3	3	25	9	9
35	14	36	6	2	40	3	3	31	3	3	33	3	9
36	1	2	1	2	4	-	1	5	3	3	3	2	7
37	1	—	—	0	4	2	3	6	2	2	4	2	6
40	593	1717	78	2	1527	215	3	1123	45	3	1331	386	9
41	3	10	0	2	5	4	3	8	3	3	7	3	9
43	42	53	2	2	43	19	3	42	17	3	44	13	9
44	845	1139	190	2	493	521	3	139	108	3	757	373	9
46	150	168	4	2	185	2	3	180	10	3	176	14	9
48	26	25	28	2	57	8	3	41	6	3	41	17	9
50	—	3	—	1	2	1	2	3	3	2	2	2	5
54	18	23	8	2	27	4	3	29	10	3	25	7	9
56	96	167	35	2	259	114	3	91	13	3	165	97	9

table 4

	SD $C1_a$			SD $C1_b$*			LE $C1_a$			LE $C1_b$		
A.A #	mean, n/M/g	sd	N	mean n/M/g	sd	N	mean nM/g	s.d.	N	mean, nM/g	s.d.	N
2	78	24	5	127	18	4	103	8	5	98	27	6
3	214	112	5	236	36	4	353	67	5	221	93	6
4	412	211	5	1352	1088	4	716	365	5	519	147	6
5	684	160	4	1121	175	4	694	83	5	3728	573	6
6	58	44	5	495	237	4	82	11	5	39	16	6
8	69	16	5	38	11	4	76	22	5	36	15	6
9	65	—	1	5	3	4	10	5	2	16	—	1
10	—	—	0	1	—	4	—	—	0	8	6	2
11	350	121	4	43	32	4	43	39	5	38	23	4
12	850	963	5	466	50	4	398	27	5	1902	416	6
13	360	539	4	412	318	4	590	119	5	607	158	5
14	796	187	5	804	216	4	1010	267	5	647	100	6
15	688	158	5	764	217	4	636	146	4	2476	548	6
17	1654	149	5	1988	349	4	1711	197	5	8090	1871	6
18	870	194	5	851	106	4	857	21	5	710	179	6
19	446	83	5	464	83	4	493	41	5	324	80	6
20	9	—	1	21	14	4	10	5	4	12	4	3
21	—	—	0	2	1	4	5	3	4	8	0	2
22	64	12	5	85	11	4	70	19	5	50	18	6
25	—	—	0	4	8	4	—	—	0	40	—	1
26	80	28	5	64	26	4	54	24	5	38	10	5
27	—	—	0	2	2	4	—	—	0	—	—	0
28	47	5	5	26	14	4	30	7	5	14	9	6
29	18	4	5	6	4	4	7	2	5	5	2	4
30	33	4	5	30	2	4	27	5	5	18	4	6
31	58	8	5	67	10	4	67	9	5	45	16	6
34	69	9	5	67	16	4	59	12	5	40	20	6
35	47	8	5	39	6	4	54	11	5	13	10	5
36	9	6	5	11	2	4	11	2	5	4	—	3
37	14	2	5	29	8	4	10	7	3	8	—	1
40	907	656	5	1517	785	4	1420	746	5	1296	464	6
41	19	9	5	26	3	4	28	9	5	13	3	5
43	147	65	4	99	34	4	138	79	5	45	35	6
44	2237	217	5	1205	177	4	3442	1600	5	688	490	6
46	235	44	5	225	43	4	244	145	5	219	72	6
48	52	8	5	55	7	4	74	19	5	57	20	6
50	21	26	5	61	22	4	20	17	3	—	—	0
54	58	10	4	80	10	4	89	38	5	47	19	6
56	129	22	5	141	12	4	147	30	5	118	53	4

* S.D. $C1_b$ is same group as Ames Control in Table 1

table 5

	LE C1 a			LE C1 b			LE O_{100-18}			LE O_{100-24}			LE O_{100-28}		
AA#	mean	sd	N	mean	sd	N	mean	sd	N	mean	sd	N	mean	sd	N
2	103	8	5	98	27	6	83	38	3	39	24	4	93	45	6
3	353	67	5	221	93	6	213	10	3	137	63	4	364	134	6
4	716	365	5	519	147	6	465	58	3	388	177	4	709	159	6
5	694	83	5	3728	573	6	533	45	3	1626	830	4	4244	949	6
6	82	11	5	39	16	6	51	5	3	12	7	4	51	15	6
8	76	22	5	36	15	6	33	23	3	42	33	5	45	7	6
9	10	5	2	16	—	1	5	3	3	176	215	2	13	14	3
10	—	—	0	8	6	2	—	—	0	12	—	1	10	6	3
11	43	39	5	38	23	4	7	4	3	34	31	2	26	16	6
12	398	27	5	1902	416	6	271	27	3	848	520	5	1702	777	6
13	590	119	5	607	158	5	496	19	3	396	162	5	604	186	6
14	1010	267	5	647	100	6	668	35	3	484	216	5	773	195	6
15	636	146	4	2476	548	6	522	18	3	1640	848	5	3560	623	6
17	1711	197	5	8090	1871	6	1394	92	3	4532	2727	5	9702	2687	6
18	857	21	5	710	179	6	749	179	3	438	212	5	915	120	6
19	493	41	5	324	80	6	431	31	3	241	108	5	453	65	6
20	10	5	4	12	4	3	6	6	3	—	—	0	14	6	5
21	5	3	4	8	—	2	2	—	1	6	3	2	4	1	2
22	70	19	5	50	18	6	49	9	3	33	19	5	70	10	3
25	—	—	0	40	—	1	—	—	0	—	—	0	—	—	0
26	54	24	5	38	10	5	48	12	3	36	20	4	44	15	6
28	30	7	5	14	9	6	25	4	3	8	3	4	28	8	6
29	7	2	5	5	2	4	5	2	2	4	—	2	8	6	6
30	27	5	5	18	4	6	27	4	3	13	7	5	28	6	6
31	67	9	5	45	16	6	55	4	3	30	13	5	63	5	6
34	59	12	5	40	20	6	39	5	3	23	14	5	70	9	6
35	54	11	5	13	10	5	40	3	3	20	11	5	57	6	6
36	11	2	5	4	—	3	4	1	3	4	—	2	10	2	4
37	10	7	3	8	—	1	3	—	1	—	—	0	12	6	2
40	1420	746	5	1296	464	6	1565	141	3	1015	443	5	1646	187	6
41	28	9	5	13	3	5	16	1	3	4	—	5	10	5	4
43	138	79	5	45	35	6	38	22	3	48	30	4	78	12	5
44	3442	1600	5	688	496	6	2063	116	3	1461	382	5	1216	91	6
46	244	145	5	219	72	6	271	31	3	175	97	5	252	33	6
48	74	19	5	57	20	6	56	7	3	36	22	5	68	16	6
50	20	17	3	—	—	0	6	5	3	—	—	0	32	12	3
54	89	38	5	47	19	6	54	9	3	35	15	5	53	23	6
56	147	30	5	118	53	4	129	29	3	80	21	3	143	23	6

mean values expressed in: nM/g

table 6

	SD C1			SD O_{100-18}			SD O_{100-50}		
AA#	mean	sd	N	mean	sd	N	mean	sd	N
2	78	24	5	76	25	8	73	11	3
3	214	112	5	313	83	8	208	7	3
4	412	211	5	638	223	8	565	89	3
5	684	160	4	686	283	8	4070	397	3
6	58	44	5	73	11	8	64	22	3
8	69	16	5	50	24	8	47	10	3
9	65	—	1	114	120	3	—	—	0
11	350	121	4	80	12	6	62	—	1
12	850	963	5	439	306	8	1839	255	2
13	360	539	4	562	244	8	507	119	3
14	796	187	5	858	115	8	725	113	3
15	688	158	5	692	107	8	3077	489	3
17	1654	149	5	1491	203	8	8559	506	3
18	870	194	5	795	141	8	704	46	3
19	446	83	5	411	71	7	445	29	3
20	9	—	1	6	4	7	8	3	2
21	—	—	0	1	—	1	4	1	2
22	64	12	5	63	7	8	57	5	3
25	—	—	0	—	—	0	3	—	1
26	80	28	5	68	79	8	23	13	3
27	—	—	0	14	13	2	—	—	0
28	47	5	5	34	7	8	24	8	3
29	18	4	5	6	3	7	8	2	3
30	33	4	5	81	147	7	23	2	3
31	58	8	5	62	6	8	51	5	3
34	69	9	5	66	14	8	51	12	3
35	47	8	5	48	3	8	41	4	3
36	9	6	5	12	3	7	10	3	3
37	14	2	5	10	9	6	15	9	3
40	907	656	5	277	65	8	1586	135	3
41	19	9	5	15	5	8	13	4	3
43	147	65	4	128	79	8	44	36	3
44	2237	217	5	2320	773	8	1521	188	3
46	235	44	5	262	39	8	210	13	3
48	52	8	5	62	8	8	62	5	3
50	21	26	5	26	23	8	38	19	3
54	58	10	4	62	15	8	65	11	3
56	129	22	5	150	28	8	146	12	3

table 7

amino acid #	S.D. control N	mean	s.d.	L.E. control N	mean	s.d.	d.f.	s	't'	P
2	9	99	32	11	100	20	18	26.025	.038	
3	9	228	83	11	280	106	18	96.457	2.674	0.02
4	9	829	844	11	608	275	18	598.838	.369	
5	8	931	283	11	2348	1637	17	1268.586	1.140	
6	9	251	274	11	58	27	18	183.771	1.050	
8	9	54	22	11	53	28	18	25.508	.039	
9	4	4	3	4	9	6	6	4.743	1.054	
11	9	181	177	9	40	31	16	127.062	1.109	
12	9	678	711	11	1218	839	18	784.693	.688	
13	8	365	411	10	598	135	16	290.093	.803	
14	9	798	191	11	811	265	18	235.005	.055	
15	9	721	178	10	1739	1039	17	765.781	1.329	
17	9	1802	237	11	5190	3587	18	2680.913	1.263	
18	9	861	153	11	776	152	18	152.445	.557	
19	9	400	151	11	400	110	18	129.830	.000	
20	5	18	13	7	10	5	10	9.088	.880	
21	4	1	1	6	5	4	8	3.221	1.241	
22	9	73	17	11	58	21	18	19.324	.776	
25	1	16	0	2	20	28	1	28.000	.142	
26	9	72	29	10	45	20	17	24.648	1.095	
27	3	2	2	0	0	0	—	—	—	
28	9	37	15	11	21	11	18	12.931	1.237	
29	9	12	8	11	5	2	18	5.537	1.264	
30	9	31	4	11	22	6	18	5.206	1.728	0.2
31	9	61	12	11	54	8	18	9.977	.701	
34	9	67	14	11	48	19	18	16.960	1.120	
35	9	43	7	10	33	23	17	17.410	.574	
36	9	9	4	8	8	4	15	4.000	.250	
37	9	20	10	5	7	6	12	8.869	1.465	
40	9	1177	742	11	1352	579	18	656.460	.266	
41	9	21	9	11	18	11	18	10.159	.295	
43	8	122	55	11	87	74	17	66.833	.523	
44	9	1777	576	11	1939	1792	18	1389.781	.116	
46	9	229	45	11	230	106	18	84.511	.011	
48	9	53	8	11	64	21	18	16.536	.665	
50	9	38	31	3	19	17	10	28.750	.660	
54	8	58	16	11	65	36	17	29.457	.101	
56	9	133	22	9	134	41	16	32.901	.030	

table 8

	L.E. control			LE O₁₀₀ 18 hr						
amino acid #	N	mean	s.d.	N	mean	s.d.	d.f.	s	't'	P is <
2	11	100	20	3	83	38	12	23.958	.709	
3	11	280	106	3	213	10	12	96.850	.691	
4	11	608	275	3	465	58	12	252.153	.567	
5	11	2348	1637	3	533	45	12	1494.482	1.214	
6	11	58	27	3	51	5	12	24.731	.283	
8	11	53	28	3	33	23	12	27.230	.734	
9	4	9	6	3	5	3	5	5.019	.796	
11	9	40	31	3	7	4	10	27.784	1.187	
12	11	1218	839	3	271	27	12	765.978	1.236	
13	10	598	135	3	496	19	11	122.380	.833	
14	11	811	265	3	668	35	12	242.332	.590	
15	10	1739	1039	3	522	18	11	939.842	1.294	
17	11	5190	3587	3	1394	92	12	3274.683	1.159	
18	11	776	152	3	749	179	12	156.823	.172	
19	11	400	110	3	431	31	12	101.210	.306	
20	7	10	5	3	6	6	8	5.267	.759	
21	6	5	4	1	2	0	5	4.000	.750	
22	11	58	21	3	49	9	12	19.519	.461	
25	2	20	28	—	—	—	—	—	—	
26	10	45	20	3	48	12	11	18.800	.159	
27	—	—	—	—	—	—	—	—	—	
28	11	21	11	3	25	4	12	10.173	.393	
29	11	5	2	2	5	2	11	2.000	.000	
30	11	22	6	3	27	4	12	5.715	.874	
31	11	54	8	3	55	4	12	7.483	.133	
34	11	48	19	3	39	5	12	17.464	.515	
35	10	33	23	3	40	3	11	20.843	.335	
36	8	8	4	3	4	1	9	3.558	1.124	
37	5	7	6	1	3	0	4	6.000	.666	
40	11	1352	579	3	1565	141	12	531.677	.400	
41	11	18	11	3	16	1	12	10.049	.199	
43	11	87	74	3	38	22	12	68.146	.719	
44	11	1939	1792	3	2063	116	12	1636.550	.075	
46	11	230	106	3	271	31	12	97.588	.420	
47	11	64	21	3	56	7	12	19.382	.412	
52	3	19	17	3	6	5	4	12.529	1.037	
54	11	65	36	3	54	9	12	33.068	.332	
56	9	134	41	3	129	29	10	38.897	.128	

No values significant at $P < 0.20$

table 9

	L.E. control			LE O.100 24 hr						
amino acid #	N	mean	s.d.	N	mean	s.d.	d.f.	S	't'	P%
2	11	100	20	4	39	24	13	20.990	2.906	<.02
3	11	280	106	4	137	63	13	97.770	1.462	<.20
4	11	608	275	4	388	177	13	255.739	.860	
5	11	2348	1637	4	1626	830	13	1490.079	.484	
6	11	58	27	4	12	7	13	23.918	1.923	<.10
8	11	53	28	5	42	33	14	29.515	.372	
9	4	9	6	2	176	215	4	107.625	1.551	<.20
11	9	40	31	2	34	31	9	31.000	.193	
12	11	1218	839	5	848	520	14	761.615	.485	
13	10	598	135	5	396	162	13	143.848	1.404	.20
14	11	811	265	5	484	216	14	251.974	1.297	
15	10	1739	1039	5	1640	848	13	984.186	.100	
17	11	5190	3587	5	4532	2727	14	3363.796	.195	
18	11	776	152	5	438	212	14	171.300	1.973	.10
19	11	400	110	5	241	108	14	109.432	1.452	<.20
20	7	10	5	—	—	—	—	—	—	
21	6	5	4	2	6	3	6	3.851	.259	
22	11	58	21	5	33	19	14	20.448	1.222	
25	2	20	28	—	—	—	—	—	—	
26	10	45	20	4	36	20	12	20.000	.450	
27	—	—	—	—	—	—	—	—	—	
28	11	28	11	4	8	3	13	9.754	2.050	<.10
29	11	5	2	2	4	0	11	1.906	.524	
30	11	22	6	5	13	7	14	6.301	1.428	<.20
31	11	54	8	5	30	13	14	9.695	2.475	<.05
34	11	48	19	5	23	14	14	17.716	1.411	<.20
35	10	33	23	5	20	11	13	20.086	.647	
36	8	8	4	2	4	0	8	3.741	1.069	
37	5	7	6	—	—	—	—	—	—	
40	11	1352	579	5	1015	443	14	543.625	.619	
41	11	18	11	5	4	0	14	9.296	1.506	<.20
43	11	87	74	4	48	30	13	66.483	.586	
44	11	1939	1792	5	1461	382	14	1528.218	8.292	.002
46	11	230	106	5	175	97	14	103.508	.531	
48	11	64	21	5	36	22	14	21.290		
50	3	19	17	—	—	—	—	—	—	
54	11	65	36	5	35	15	14	31.464	.953	
56	9	134	41	3	80	21	10	37.854	1.426	<.20

table 10

amino acid #	L.E. control N	mean	s.d.	LE O_{100} 26hr N	mean	s.d.	d.f.	s	't'	P
2	11	100	20	6	93	45	15	30.686	.228	
3	11	280	106	6	364	134	15	116.086	.723	
4	11	608	275	6	709	159	15	242.577	.416	
5	11	2348	1637	6	4244	949	15	1444.545	1.312	
6	11	58	27	6	51	15	15	23.685	.295	
8	11	53	28	6	45	7	15	23.216	.344	
9	4	9	6	3	13	14	5	10.000	.400	
11	9	40	31	6	26	16	13	26.264	.533	
12	11	1218	839	6	1072	777	15	818.855	.178	
13	10	598	135	6	604	186	14	155.150	.038	
14	11	811	265	6	773	195	15	246.084	.154	
15	10	1739	1039	6	3560	623	14	912.466	1.995	.10
17	11	5190	3587	6	9702	2687	15	3314.267	1.361	.20
18	11	776	152	6	915	120	15	142.136	.977	
19	11	400	110	6	453	65	15	89.861	.589	
20	7	10	5	5	14	6	10	5.422	.737	
21	6	5	4	2	4	1	6	3.674	.272	
22	11	58	21	3	70	10	12	19.600	.612	
25	2	20	28	—	—	—	—	—	—	
26	10	45	20	6	44	15	14	18.371	.054	
27	—	—	—	—	—	—	—	—	—	
28	11	21	11	6	28	8	15	10.099	.693	
29	11	5	2	6	8	6	15	3.829	.783	
30	11	22	6	6	28	6	15	6.000	1.000	
31	11	54	8	6	63	5	15	7.141	1.260	
34	11	48	19	6	70	9	15	16.360	1.344	.20
35	10	33	23	6	57	6	14	18.780	1.277	
36	8	8	4	4	10	2	10	3.521	.568	
37	5	7	6	2	12	6	5	6.000	.833	
40	11	1352	579	6	1646	187	15	484.923	.606	
41	11	18	11	4	10	5	13	9.942	.804	
43	11	87	74	5	78	12	14	62.869	.143	
44	11	1939	1792	6	1216	91	15	1464.104	.493	
46	11	230	106	6	252	33	15	88.620	.248	
48	11	64	21	6	68	16	15	19.476	.205	
50	3	19	17	3	32	12	4	14.713	.883	
54	11	65	36	6	53	23	15	32.254	.372	
56	9	134	41	6	143	23	13	35.184	.255	

table 11

	L.E. control			LE $H_2 p_2 O_{20}$ 18hr						
amino acid #	N	mean	s.d.	N	mean	s.d.	d.f.	S	't'	P<
2	11	100	20	15	29	15	24	17.260	4.113	.002
3	11	280	106	15	142	61	24	82.778	1.667	.20
4	11	608	275	14	530	268	23	271.065	.287	-
5	11	2348	1637	14	2515	1289	23	1450.599	.115	-
6	11	58	27	15	22	15	24	20.856	1.726	.10
8	11	53	28	15	77	66	24	53.550	.354	
9	4	9	6	15	126	90	17	81.712	1.431	.20
11	9	40	31	13	50	52	20	44.797	.223	
12	11	1218	839	15	1763	816	24	825.661	.660	
13	10	598	135	14	379	203	22	178.343	1.227	
14	11	811	265	15	644	281	24	274.446	.608	
15	10	1739	1039	14	1164	557	22	790.538	.727	
17	11	5190	3587	14	5886	3173	23	3359.275	.207	
18	11	776	152	15	620	265	24	224.924	.693	
19	11	400	110	15	455	206	24	172.615	.318	
20	7	10	5	11	5	2	16	3.446	1.450	.20
21	6	5	4	11	19	18	15	14.877	.941	
22	11	58	21	15	36	18	24	19.306	1.139	-
25	2	20	28	—	—	—	—	—	—	-
26	10	45	20	15	28	19	23	19.397	.876	-
27	—	—	—	—	—	—	—	—	—	-
28	11	21	11	15	14	8	24	9.367	.747	-
29	11	5	2	10	6	5	19	3.734	.267	-
30	11	22	6	14	15	8	23	7.199	.972	-
31	11	54	8	14	38	18	23	14.524	1.101	-
34	11	48	19	15	46	23	24	21.424	.093	-
35	10	33	23	15	32	16	23	19.047	.052	-
36	8	8	4	8	6	2	14	3.162	.632	-
37	5	7	6	—	—	—	—	—	—	-
40	11	1352	579	14	1317	748	23	679.704	.051	-
41	11	18	11	10	8	6	19	8.985	1.112	-
43	11	87	74	14	68	38	23	56.542	.330	
44	11	1939	1792	14	1756	1074	23	1431.141	.127	
46	11	230	106	14	192	108	23	107.135	.354	
48	11	64	21	14	39	21	23	21.000	1.190	
50	3	19	17	6	35	32	7	28.530	.560	
54	11	65	36	13	39	20	22	28.412	.915	
56	9	134	41	13	73	29	20	34.307	1.778	.10

table 12

	SD control			SD O100 18 hr.						
amino acid #	N	mean	s.d.	N	mean	s.d.	d.f.	s	't'	P <
2	9	99	32	8	76	25	15	28.944	.794	
3	9	228	83	8	313	83	15	83.000	.301	
4	9	829	844	8	638	223	15	634.916	.300	
5	8	901	283	8	686	283	14	283.000	.759	
6	9	251	274	8	73	11	15	200.242	.888	
8	9	54	22	8	50	24	15	22.955	.174	
9	4	4	3	3	114	120	5	75.930	1.448	
11	9	181	177	6	80	12	13	139.049	.726	
12	9	678	711	8	439	306	15	559.739	.426	
13	8	365	411	8	562	244	14	337.977	.582	
14	9	798	191	8	858	115	15	160.088	.374	
15	9	721	178	8	692	107	15	149.134	.194	
17	9	1802	297	8	1491	203	15	257.440	1.208	
18	9	861	153	8	795	141	15	147.521	.447	
19	9	400	151	7	411	71	14	123.245	.089	
20	5	18	13	7	6	4	10	8.786	1.365	
21	4	1	1	1	1	0	3	1.000	.000	
22	9	73	17	8	63	7	15	13.304	.751	
25	1	16	0	—	—	—	—	—	—	
26	9	72	29	8	68	79	15	57.974	.068	
27	3	2	2	2	14	13	3	7.681	1.562	
28	9	37	15	8	34	7	15	11.952	.251	
29	9	12	8	7	6	3	14	6.358	.943	
30	9	31	4	7	81	147	14	96.281	.519	
31	9	61	12	8	62	6	15	9.674	.103	
34	9	67	14	8	66	14	15	14.000	.071	
35	9	43	7	8	48	3	15	5.507	.907	
36	9	9	4	7	12	3	14	3.605	.832	
37	9	20	10	6	10	9	13	9.627	1.038	
40	9	1177	742	8	277	65	15	543.696	1.655	
41	9	21	9	8	15	5	15	7.407	.810	
43	8	122	55	8	128	79	14	68.066	.088	
44	9	1777	576	8	2320	773	15	675.125	.804	
46	9	229	45	8	262	39	15	42.306	.780	
48	9	53	8	8	62	8	15	8.000	1.125	
50	9	38	31	8	26	23	15	27.557	.435	
54	8	68	16	8	62	15	14	15.508	.386	
56	9	133	22	8	150	28	15	24.979	.680	

No values significant at $P < 0.20$

table 13

	SD control			SD O_{100} 50 hr						
amino acid #	N	mean	s.d.	N	mean	s.d.	d.f.	S	't'	P is <
2	9	99	32	3	73	11	10	29.041	.895	
3	9	228	83	3	208	7	10	74.303	.269	
4	9	829	844	3	565	89	10	755.945	.349	
5	8	901	283	3	4070	397	9	311.954	10.158	.002
6	9	251	274	3	64	22	10	245.270	.762	
8	9	54	22	3	47	10				
9	4	4	3	—	—	—	—	—	—	
11	9	181	177	1	62	0	8	177.000	.672	
12	9	678	711	2	1839	255	9	675.704	1.718	.20
13	8	365	411	3	507	119	9	366.783	.387	
14	9	798	191	3	725	113	10	178.153	.409	
15	9	721	178	3	3077	489	10	270.502	8.709	.002
17	9	1802	297	3	8559	506	10	348.961	19.363	.002
18	9	861	153	3	704	46	10	138.384	1.134	
19	9	400	151	3	445	29	10	135.679	.331	
20	5	18	13	2	8	3	5	11.704	.854	
21	4	1	1	2	4	1	4	1.000	3.000	.05
22	9	73	17	3	57	5	10	15.368	1.041	
25	1	16	0	1	3	0	—	—	—	
26	9	72	29	3	23	13	10	26.581	1.843	.10
27	3	2	2	—	—	—	—	—	—	
28	9	37	15	3	24	8	10	13.885	.936	
29	9	12	8	3	8	2	10	7.211	.554	
30	9	31	4	3	23	2	10	3.687	2.169	.10
31	9	61	12	3	51	5	10	10.963	.912	
34	9	67	14	3	51	12	10	13.623	1.174	
35	9	43	7	3	41	4	10	6.511	.307	
36	9	9	4	3	10	3	10	3.820	.261	
37	9	20	10	3	15	9	10	9.808	.509	
40	9	1177	742	3	1586	135	10	666.405	.613	
41	9	21	9	3	13	4	10	8.246	.970	
43	8	122	55	3	44	36	9	51.388	1.517	.20
44	9	1777	576	3	1521	188	10	522.005	.490	
46	9	229	45	3	210	13	10	40.666	.467	
48	9	53	8	3	62	5	10	7.496	1.200	
50	9	38	31	3	38	19	10	29.000	.000	
54	8	68	16	3	65	11	9	15.033	.199	
56	9	133	22	3	146	12	10	20.396	.637	

table 14

	LE control			Fluothane total						
amino acid #	N	mean	s.d.	N	mean	s.d.	d.f.	s	't'	P is <
2	11	100	20	9	27	13	18	17.243	4.233	.002
3	11	280	106	9	133	38	18	82.969	1.771	.10
4	11	608	275	9	282	94	18	214.338	1.520	.20
5	11	2348	1637	9	2321	644	18	1293.478	.133	
6	11	58	27	9	24	11	18	21.419	1.587	.20
8	11	53	28	9	20	10	18	21.908	1.506	.20
9	4	9	6	6	9	2	8	4.000	.000	
11	9	40	31	—	—	—	—	—	—	
12	11	1218	839	9	857	572	18	732.449	.492	
13	10	598	135	8	342	168	16	150.331	1.702	.20
14	11	811	265	9	545	115	18	211.876	1.255	
15	10	1739	1039	9	2519	469	17	821.597	.949	
17	11	5190	3587	9	5246	2277	18	2677.871	.020	
18	11	776	152	9	639	124	18	140.247	.976	
19	11	400	110	9	299	38	18	85.813	1.176	
20	7	10	5	7	28	26	12	18.721	.961	
21	6	5	4	5	2	1	9	3.054	.982	
22	11	58	21	8	41	18	17	19.819	.857	
25	2	20	28	—	—	—	—	—	—	
26	10	45	20	8	25	7	16	15.698	1.274	
27	—	—	—	—	—	—	—	—	—	
28	11	21	11	8	21	10	17	10.599	.000	
29	11	5	2	7	5	3	16	2.423	.000	
30	11	22	6	8	24	7	17	6.430	.311	
31	11	54	8	8	62	16	17	11.960	.668	
34	11	48	19	9	25	9	18	15.380	1.495	.20
35	10	33	23	9	33	3	17	16.861	.000	
36	8	8	4	7	3	2	13	3.234	1.546	.20
37	5	7	6	6	4	2	9	4.268	.702	
40	11	1352	579	9	1331	386	18	502.459	.041	
41	11	18	11	9	7	3	18	8.439	1.303	
43	11	87	74	9	44	13	18	55.833	.770	
44	11	1939	1792	9	757	373	18	1358.628	.869	
46	11	230	106	9	176	14	18	79.557	.678	
48	11	64	21	9	41	17	18	19.324	1.190	
50	3	19	17	5	2	2	6	9.949	1.708	.20
54	11	65	36	9	25	7	18	27.235	1.468	.20
56	9	134	41	9	165	97	16	74.404	.416	